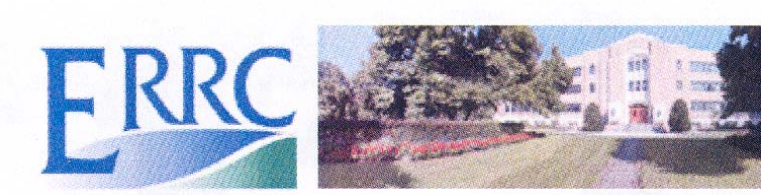

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Thermal Resistance of *Listeria monocytogenes, Salmonella* Heidelberg, and *Escherichia coli* O157:H7 at Elevated Temperatures†

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ABSTRACT

A continuous-flow apparatus was developed to measure thermal resistance (*D*- and *z*-values) of microorganisms at temperatures above 65°C. This apparatus was designed to test whether vegetative microorganisms exhibited unusually high thermal resistance that prevented them from being completely eliminated at temperatures applicable to vacuum-steam-vacuum processes (116 to 157°C). The apparatus was composed of a high-pressure liquid chromatography pump, a heating unit, and a cooling unit. It was designed to measure small *D*-values (<1 s). Three randomly selected organisms, *Listeria monocytogenes, Salmonella* Heidelberg, and *Escherichia coli* O157:H7 suspended in deionized water were tested in the continuous-flow apparatus at temperatures ranging from 60 to 80°C. Studies showed that the *D*-values of these organisms ranged from 0.05 to 20 s. Heating at 80°C was found to be basically the physical limit of the system. Experimental results showed that *L. monocytogenes, Salmonella* Heidelberg, and *E. coli* O157:H7 did not exhibit unusual heat resistance. The conditions used in the vacuum-steam-vacuum processes should have completely inactivated organisms such as *L. monocytogenes, Salmonella* Heidelberg, and *E. coli* O157:H7 if present on food surfaces. The complete destruction of bacteria during vacuum-steam-vacuum processes might not occur because the surface temperatures never reached those of the steam temperatures and because bacteria might be hidden beneath the surface and was thus never exposed to the destructive effects of the steam.

Thermal processing has been one of the most effective food processing technologies for eradicating foodborne pathogens. For long-term storage, high-temperature (>121.1°C or 250°F) thermal processing has been successfully used in the food industry to destroy spore formers, particularly *Clostridium botulinum,* in low-acid foods. For ready-to-eat (RTE) meat and poultry products, low-temperature cooking has been used to kill many common foodborne pathogens, including *Listeria monocytogenes, Salmonella,* and *Escherichia coli* O157:H7. These products are usually packaged with plastic materials after thermal processing to prevent further contamination and are kept under refrigeration.

RTE meat and poultry products are fully cooked and should be free of pathogens. However, many incidents of food poisoning have been reported in recent years to link foodborne pathogens, particularly *L. monocytogenes,* to RTE meat and poultry products *(1–3).* This organism is potentially dangerous to consumers with compromised immune systems and who are young, old, or pregnant. Thermal processes used in the food industry are designed to eliminate *L. monocytogenes* from RTE meat and poultry products. However, cross-contamination can occur. According to a joint study conducted in a turkey franks production facility, scientists from the U.S. Centers for Disease Control and Prevention and the U.S. Department of Agriculture identified that the peeling operation immediately after thermal processing was primarily responsible for the recontamination of fully cooked turkey franks by *L. monocytogenes* before final packaging *(15).* As a result, many RTE meat and poultry products potentially contaminated with *L. monocytogenes* might enter the market, causing outbreaks of foodborne listeriosis among high-risk populations.

Cross-contamination after thermal processing usually occurs on the surface of cooked products. Steam surface pasteurization has been proposed to remedy this problem. A new process was developed with the use of a combination of vacuum and saturated steam to rapidly destroy the bacteria attached onto food surfaces *(5, 10).* This process involves a sequence of treatments with vacuum and steam. It starts with the placement of a food material in an enclosed chamber. In the first step, vacuum is applied to remove air and moisture on and surrounding the food surface. In the second step, saturated steam is flushed into the chamber. The thermal energy released from the saturated steam raises the surface temperature of the food and subsequently kills bacteria attached onto it. In the final step, vacuum is applied again to remove the heat from the food surface. These three steps, vacuum-steam-vacuum (VSV), form a treatment cycle. This technology was initially developed to treat highly heat-sensitive materials such as raw chicken meats *(10–12)* and was later adopted to treat other foods such as hot dogs, catfish, fruits, and vegetables *(6, 8, 9).*

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

In the studies reported by Morgan et al. *(11, 12)*, small chicken meat samples (~5 g) inoculated with *Listeria innocua* (~10^7) on the surface were treated with cycles of vacuum and steam at temperatures ranging from 126 to 157°C. The objective of their research was to develop a process that could effectively kill pathogens attached onto the surface of heat-sensitive foods such as raw chicken meat without inducing thermal damage. Although various treatment times were used at each steam temperature, the results of VSV treatments were consistent. A range of 2- to 4-log reduction was observed. Treatment with a steam temperature of 138°C for 26 ms showed the highest kill (4 log). Further increasing the steam temperature to 149 or 157°C for 26 ms did not lead to improved bacteria destruction. Instead, less kill was observed at higher temperatures. These observations raised an interesting question as to the mechanism of bacterial kill during the VSV process. Because the steam temperatures used in the studies were very high, one would expect that none of the vegetative bacterial cells could survive the severe heating process. Yet substantial amounts of bacteria survived the VSV process.

A larger scale VSV device capable of treating a whole chicken was developed *(5, 7)*. Tests were conducted on chicken drumsticks, fryers cut in half, and whole or half Cornish hens. Samples treated with saturated steam at temperatures ranging from 116 to 157°C for up to 2 s showed little difference in aerobic plate counts (APC), *E. coli*, and coliform counts when compared with controls. Similar results were observed in subsequent tests in the VSV unit modified with mandrels so that steam could be injected directly into the visceral cavities of chicken carcasses. Decreased counts (ca. 0.7 to 0.8 log CFU/ml) were observed for chickens surface inoculated with 4.8 log CFU/ml of *L. innocua (6)*. Tests were also conducted to treat catfish in the VSV unit *(8)*. At 138, 143, and 149°C for 0.1 s, the decreases in bacterial counts also ranged between 0.7 and 0.8 log CFU/ml for APC with an initial load of approximately 3.2 log CFU/ml. Even under optimized conditions (143°C and four cycles), an approximate 2-log reduction in the bacterial count was achieved for catfish surface inoculated with *L. innocua* (initial load = 5 log CFU/ml). Statistical analysis showed that heating time did not significantly affect the bacterial kill. A study of ham inoculated with *L. innocua* on the surface and treated at 138°C steam (0.1 s each cycle) for 2 to 3 cycles showed that the decreases in bacterial counts were less than 3 log CFU per sample, with an initial approximately 8-log bacterial load *(14)*. The best results were observed in the treatment of hot dogs *(7)*, in which close to 4 to 5 log CFU/ml of *L. innocua* could be achieved. However, the total steam treatment time was extended to 0.3 to 0.4 s at 138°C for 2 to 4 cycles. With an initial bacterial load of 5.2 log CFU/ml, some *L. innocua* survived the steam treatment under these severe conditions. For various fruits and vegetables (e.g., papayas, mangoes, avocados, kiwis, carrots, and cucumbers) surface inoculated with approximately 5 log CFU/ml of *L. innocua* and treated at 138 or 143°C for 0.1 s, bacteria (1.4 to 1.6 log CFU/ml) still survived the heating *(9)*.

In all reports regarding VSV, steam treatment conditions at temperatures above 116°C were fairly severe for vegetative cells; therefore, theoretically none of them should have survived. Increased heating time should have improved the bacterial kill, and yet experimental results proved otherwise. One possibility is that vegetative cells might exhibit unusually high thermal resistance at elevated temperatures such that bacteria could withstand the severe heating. Therefore, the objective of this study was to measure the thermal resistance (*D*- and *z*-values) of several randomly selected vegetative microorganisms at elevated temperatures. Because vegetative bacterial cells at temperatures above 65°C might be sensitive to heat, a new apparatus was developed to measure their *D*- and *z*-values.

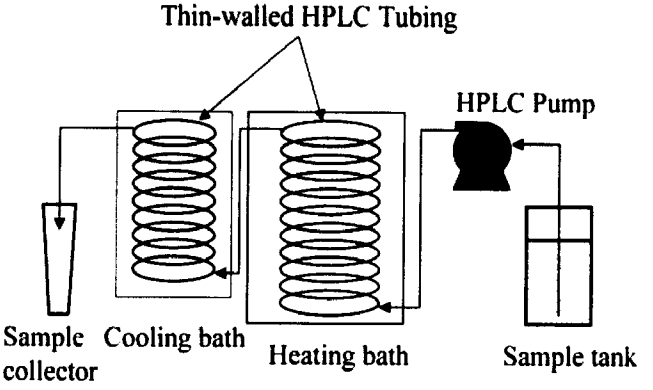

FIGURE 1. *A continuous-flow apparatus for investigating thermal resistance of microorganisms at elevated temperatures.*

MATERIALS AND METHODS

Development of a continuous-flow heating apparatus. To study the thermal resistance of vegetative bacteria, a continuous-flow heating apparatus was developed (Fig. 1). The apparatus consisted of a precision high-pressure liquid chromatography (HPLC) pump, a heating unit, and a cooling unit. The HPLC pump drew a refrigerated bacterial solution from a sample tank and fed it to the heating unit, which was a thin-walled stainless steel coil fully submerged in a recirculating water bath (Fisher Scientific IsoTemp Model 1016S, Pittsburgh, Pa.). The effluent from the heating unit was immediately fed to the cooling unit, consisting of a thin-walled stainless steel coil fully submerged in iced water in a 2-liter beaker. Heating and cooling coils and the coils connecting the system were standard HPLC tubing: $\frac{1}{16}$-in. (0.1588-cm) external diameter and 5 ft (152.4 cm) in length. The cooling coil and the tubing used to connect the HPLC pump and the heating coil were 0.03-in. (0.0762-cm) internal diameter (i.d.) and 5 ft in length. Depending on the heating temperature, the heating coil was selected from a collection of tubings with different i.d.s (0.007, 0.01, 0.02, 0.03, and 0.0525 in.). With sufficiently long heating and cooling coils, it was assumed that the bacterial solution was immediately heated to the temperature of the hot water bath as it was pumped to the heating unit and cooled to the temperature of the iced water after it was fed into the cooling unit.

A German-made HPLC pump (Knauer HPLC pump 64) was calibrated and used in this study. The maximum capacity of the pump was 10 ml/min, and the flow rate could be adjusted from 0 to 10 ml/min to the accuracy of 0.1 ml/min. With a fixed coil length and i.d., the residence time of the bacterial suspension could be regulated easily by adjusting the speed of the pump.

Calculation of residence time. The residence time of the bacterial solution can be determined from equation 1,

$$t = 1.5 \times 10^7 \frac{\pi D^2 L}{V} \quad (1)$$

where t is the overall residence time (s), V is the volumetric flow rate (ml/min), L is the length of the heating coil fully submerged in the water bath (m), and D is the internal diameter of the heating coil (m).

The flow pattern can be calculated from the Reynolds number (Re) of the flow system (equation 2),

$$\mathrm{Re} = \frac{Du\rho}{\mu} = \frac{\rho V \times 10^7}{1.5\pi\mu D} \quad (2)$$

where u is the velocity (m/s), μ is the viscosity (Pa·s), and ρ is the density (kg/m^3) of the bacterial solution.

With pure water as a reference, the Reynolds numbers of the fluid flow in the heating coils of different internal diameters remained mostly in laminar and transitional regions, except for the flows in the tubing with 0.007-in. i.d. and at temperatures >100°C. At temperatures below 80°C, almost all flows were laminar.

The velocity profile of a laminar flow in a straight pipe across the diameter is parabolic. The fluid traveling at the center can pass through the system twice as fast as the bulk fluid. The bacteria flowing along the axis might receive less heating than the bulk fluid. It would be desirable to achieve turbulent flow in the flow system; however, it would require a high-pressure, high-flow-rate positive pump to deliver a small amount of fluid through a small diameter in order to achieve instantaneous heating. The pressure drop in the system is greater than 10 MPa. It was difficult to find such a powerful pump that is still small enough for this application. An HPLC pump was the only choice available for this study. Therefore, although imperfect, the average residence time for the bulk fluid, determined from equation 1, was used to present the overall heating time that the bacteria received in the heating coil.

Organisms. Three typical foodborne pathogens, *L. monocytogenes* (Scott A, ATCC 49594), *Salmonella* Heidelberg (strain F5038BG1), and *E. coli* O157:H7 (strain C1-9218), were used in this study. The *L. monocytogenes* strain was obtained from Dr. Christopher Sommers of the Agricultural Research Service, Eastern Regional Research Center (ARS-ERRC); *Salmonella* Heidelberg and *E. coli* were obtained from Dr. Vijay Juneja of ARS-ERRC. These organisms were arbitrarily chosen for this study from a collection of stock cultures.

The initial culture of each bacterial strain was propagated in a 50-ml brain heart infusion broth (Difco/BD, Sparks, Md.) at 37°C for 24 h. The culture was then maintained in tryptic soy agar (TSA; Difco/BD) slants and stored at 4°C. New slants of bacterial cultures were prepared every 2 weeks.

For each experiment, a loopful of each culture was transferred to 10 ml of brain heart infusion broth and incubated for 24 h at 37°C in an orbital shaker (medium speed). Each culture was centrifuged (2,400 × g) at 4°C for 15 min and washed twice with 10 ml of sterile 0.1% peptone water (wt/vol). Bacterial cell pellets of each strain were resuspended in 10 ml of 0.1% peptone water. To prepare for a bacterial solution, a 3-ml aliquot of each strain was dispersed in approximately 200 ml of sterile deionized water in a 250-ml beaker. Once prepared, the bacterial solution was immediately used for heat inactivation tests.

Heat inactivation. The heating coil was submerged in the water bath and heated to a predetermined temperature. The system was first flushed with 70% ethanol solution for approximately 20 min and then with sterile deionized water for 10 to 15 min, at which time the system was ready for heat inactivation tests.

The bacterial solution prepared previously was placed on ice and fed into the system. The residence time was controlled by adjusting the configuration of the heating coil (i.d. and length) and the flow rate of the bacterial solution. Before an experiment, the heating coil length (minimum 50 cm, maximum 304.8 cm) was determined, on the basis of the heating temperature and the coil i.d. The heating coil configuration was predetermined such that at least a 5-log reduction could be achieved in an experiment. For *L. monocytogenes*, the heating temperatures were 60, 62, 66, 70, 74, 76, and 80°C. For *Salmonella* Heidelberg, the heating temperatures were 68, 70, 72, 74, 76, and 78°C, whereas for *E. coli* O157:H7, the heating temperatures were 68, 70, 72, 74, and 76°C.

During each heating experiment, the flow rate was set at 1.8, 2.2, 3.0, 4.5, and 9.0 ml/min to vary the residence time. The experiment started with 1.8 ml/min (the longest heating time, most kill) and progressed to 9.0 ml/min (the shortest heating time, least kill). At each adjustment of the flow rate, the system was flushed with the bacterial solution for at least 5 min before the effluent (3 to 4 ml) was collected. Effluents were immediately placed in ice after being collected. During the experiment, ice was continuously added to the cooling unit. Three independent experiments were conducted to obtain the thermal resistance of bacteria at a constant temperature.

Enumeration of surviving bacteria. The effluents were serially diluted with 0.1% sterile peptone water and then hand-plated onto the surface of TSA plates. After 120 min of resuscitation at room temperature to allow for recovery of heat-damaged cells, the TSA plates were incubated at 37 ± 1°C for 30 to 48 h. An average of three plate readings for each sample was used to represent the number of bacteria surviving after heat inactivation. The bacterial counts were converted to log CFU per milliliter and used to calculate the D-values of the organisms.

Determination of D- and z-values. Survival curves were constructed by plotting the log bacterial counts against heating time for each temperature. D-values were calculated from the linear portion of each survival curve (equation 3). The z-value of each organism was calculated by plotting the log of D-values against the heating temperature (equation 4).

$$\log C = \log(C_0) - \frac{t}{D} \quad (3)$$

$$\log D = \log(D_0) - \frac{T}{z} \quad (4)$$

RESULTS AND DISCUSSION

Figure 2 shows typical survival curves for *L. monocytogenes*, *Salmonella* Heidelberg, and *E. coli* O157:H7. Most survival curves were linear with only a few showing a small lag during heating. Even with the continuous-flow system, the highest achievable test temperature for *L. monocytogenes*, *Salmonella* Heidelberg, and *E. coli* O157:H7 was 80, 78, and 76°C, respectively. At higher temperatures, no survivors were observed, indicating that these organisms are not particularly heat resistant and can be killed easily. The average D-value for *L. monocytogenes* at 80°C, *Salmonella* Heidelberg at 78°C, and *E. coli* O157:H7 at 76°C was 0.05, 0.06, and 0.11 s, respectively. Because the residence time is proportional to the length and the square of the i.d. of the heating coil, it is possible to measure D-values at higher temperatures, provided a smaller and shorter tubing is used. The smallest i.d. and length used in this

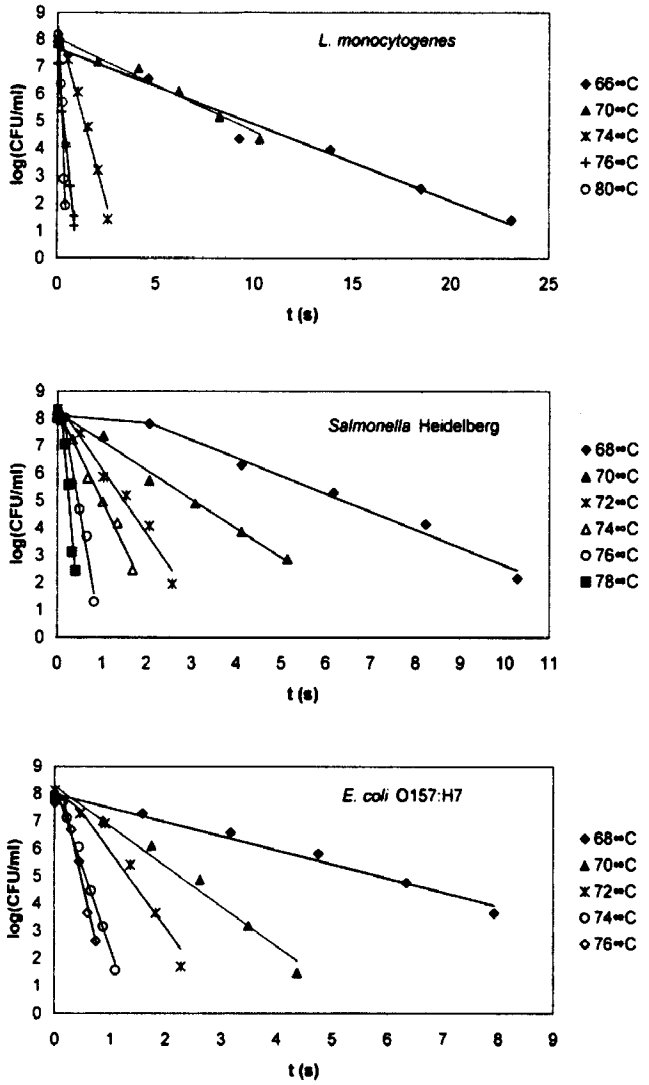

FIGURE 2. *Representative survival curves of* L. monocytogenes, Salmonella *Heidelberg, and* E. coli *O157:H7 obtained from the continuous-flow apparatus.*

study was 0.007 in. (0.0178 cm) and 50 cm, respectively. This combination was found to be practically the limit of the continuous-flow heating apparatus for *L. monocytogenes, Salmonella* Heidelberg, and *E. coli* O157:H7 in this study.

The lowest temperature tested in this study was 60°C for *L. monocytogenes.* The average *D*-value of *L. monocytogenes* at 60°C was 20.5 s. This value was measured with a heating coil of 0.0525 in. (0.133 cm) i.d. and a length of 10 ft (304.8 cm). It was also possible to conduct heat inactivation tests at lower temperatures. However, a much longer heating coil would have to be used. For measuring larger *D*-values, other techniques, such as the submerged coil method, can be used *(4).*

Table 1 lists the regression coefficients of equation 4 for three organisms. As expected, *L. monocytogenes* was the most heat-resistant among the three, with *E. coli* O157:H7 being the most sensitive to heat.

Because the *D*- and *z*-values of these organisms were obtained while in water, they cannot be directly applicable to meats because the presence of protein and fat usually provides some protective effects to microorganisms. Murphy et al. *(13)* used *L. innocua* as a surrogate for *L. monocytogenes* and measured its thermal resistance in chicken breast meat. The highest temperature used in their study was 70°C. Extrapolation must be used to obtain the *D*-values at higher temperatures. A comparison of the *D*-value of *L. innocua* in chicken breast meat *(13)* with *L. monocytogenes* in water from this study can be seen in Figure 3. The extrapolated *D*-values of *L. innocua* in chicken breast meat were lower than those of *L. monocytogenes* in water at temperatures above 100°C. Because it is practically impossible to measure the *D*-values of vegetative microorganisms in solid foods because of the slow rate of heat transfer and the difficulty of sampling at high temperatures (>70°C), the *D*- and *z*-values obtained from water can be used to make some scientifically reasonable inferences.

The lowest temperature used by Morgan et al. *(11)* was 127°C for 26 ms on fresh chicken meat. The authors achieved a 2.2-log reduction for *L. innocua* inoculated onto the meat surface. The *D*-value of *L. monocytogenes* at 127°C, extrapolated from Table 1, is 3.62×10^{-5} ms. The steam treatment at 127°C for 26 ms is severe even for *L. monocytogenes,* which is conventionally considered more heat resistant than *L. innocua* in meats. The heating condition at 127°C for 26 ms, equivalent to a process to produce a 7.2×10^5-log reduction for *L. monocytogenes,* should have completely eliminated *L. innocua* on chicken. Even if the thermal resistance of *L. innocua* in chicken was hypothetically 10^5 times higher than *L. monocytogenes* in water (which is unlikely), this process should have resulted in approximately a 7-log reduction in bacterial counts. Instead, only a 2- to 4-log reduction was consistently observed, regardless of the temperature and steam time. For example, applying even more severe heat at 157°C for 26 ms, equivalent to a 2.0×10^7-log reduction for *L. monocytogenes,* achieved only a 2.7-log reduction in *L. innocua* on chicken meat. Similar observations can be found in the work conducted by Kozempel et al. *(6–9).* Under the extremely severe heating conditions of VSV, survival of many types of microorganisms, including *L. innocua, E. coli,* or

TABLE 1. *Regression coefficients of equation 4 for* L. monocytogenes, Salmonella *Heidelberg, and* E. coli *O157:H7*[a]

Organism	log D_0	1/z	z-value (°C)	R^2
L. monocytogenes	9.32 (0.37)	0.132 (0.005)	7.57 (0.30)	0.97
Salmonella Heidelberg	9.82 (0.43)	0.142 (0.006)	7.06 (0.30)	0.97
E. coli O157:H7	10.57 (0.50)	0.152 (0.007)	6.56 (0.30)	0.98

[a] Values are means (standard errors).

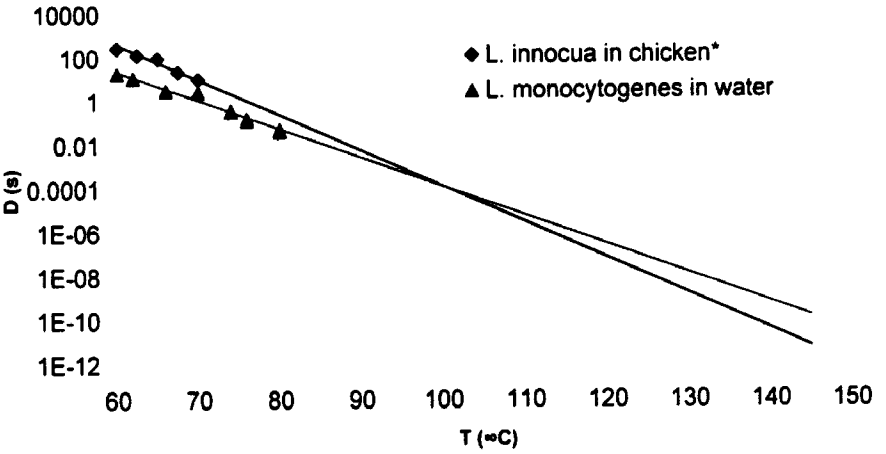

FIGURE 3. *Comparison of* D-*values of* L. innocua *in chicken meat and* L. monocytogenes *in water. Data were extrapolated values for* L. innocua *above 70°C, and for* L. monocytogenes *above 80°C.* * D-*values of* L. innocua *were adopted from Murphy et al. (13).*

natural flora, was observed in various meats, fruits, or vegetables. According to Kozempel et al. *(7),* an ultrahigh temperature (138°C) was applied to treat hot dogs. The total steam treatment times were 0.3 and 0.4 s, with 0.1 s for each cycle; reductions in bacterial counts were between 4 and 5 log CFU/ml. Considering that the *D*-value of *L. monocytogenes* at 138°C, extrapolated from Table 1, is in the magnitude of only 1.28×10^{-6} ms, the VSV process was extremely severe. Because *Salmonella* and *E. coli* are less heat resistant than *L. monocytogenes,* none of them should have survived.

Two possible hypotheses could explain the incomplete destruction of bacteria during VSV processes. In the first, the surface temperatures of the foods treated by VSV processes might never reach steam temperatures. In the second, because no food is perfectly smooth, bacteria could be hidden within the cavities underneath the surface of foods where steam cannot reach. Heat has to be transferred by conduction in order to raise the temperature in cavities. Because the VSV steam treatment time in a cycle is extremely short (<0.1 s), there is not sufficient time for heat to be conducted to these areas. Consequently, the cavity areas remain "cold" no matter how many cycles are used. As a result, large numbers of bacteria could survive. That the effectiveness of VSV processes was independent of temperature in all the VSV studies *(6–8, 11, 12)* could be a result of bacteria hidden beneath the surface. A more probable explanation to the incomplete destruction of bacteria during VSV might be the combination of both hypotheses—that the surface temperature is never instantaneously raised to the steam temperature and that bacteria are hidden beneath the surface. These hypothetical explanations can only be verified by directly measuring the time-temperature history on and immediately beneath the surfaces of foods treated by VSV processes. One potential solution for improving the effectiveness of VSV processes might be to substantially increase the steam time. A substantially longer heating time would allow the surface temperature to reach those of saturated steam and also allow heat to penetrate deeper into the solid foods so that bacteria immediately underneath the surface could be heated to a lethal temperature. Consequently, a complete destruction of surface-contaminated bacteria in ready-to-eat foods potentially can be achieved.

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